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L3: Entry 13 of 557 File: USPT Nov 6, 2001

DOCUMENT-IDENTIFIER: US 6313330 B1
TITLE: Processes of selectively separating and purifying eicosapentaenoic and docoshexaenoic acids or their esters

Brief Summary Paragraph Right (12):
The diatomaceous earth can be usually used in its uncalcined or calcined form, but the calcined earth is preferable. The calcined diatomaceous earth can be produced by crushing a raw diatomaceous earth, drying the crushed product, subjecting it to repeated grinding and classification operations to remove impurities, calcining the classified product at high temperature, and further subjecting it to repeated grinding and classification operations to adjust the particle size. It is preferable that the diatomaceous earth has a pore size of 0.1-10 .mu.m, a specific surface area of 0.5-50 m.sup.2 /g and a pore volume of 1-10 ml/g for increasing the ability to hold an aqueous silver salt solution and securing good contact with highly unsaturated fatty acids or their derivatives to be treated. It is more preferable that the diatomaceous earth has a bulk density of 0.1-0.3 g/ml, taking the size of column into consideration. The diatomaceous earth which can be used in the present invention is commercially available, but not limited to a specified one, a typical example of which is "Extrelut.RTM. 13076"available from Merck Co., Ltd.

Brief Summary Paragraph Right (22):
In the present invention, the pore volume of the diatomaceous earth is 1-10 ml/g, which indicates that the amount of the aqueous medium held per gram of the diatomaceous earth is 1-10 ml. For instance, when silver is carried in the form of an aqueous silver nitrate solution, silver nitrate forms its saturated solution with water of about half amount based on the weight of silver nitrate. Accordingly, only 50% of the pore volume of the diatomaceous earth is used even if silver nitrate of the same weight as the diatomaceous earth is held in the form of the aqueous solution. This can inhibit an effusion of silver. That is, two-fold amounts of the diatomaceous earth based on the aqueous medium in the aqueous silver nitrate solution can be used, which results in no effusion of the silver salt from the column when the extraction is carried out with an organic solvent such as hexane or the like.

Detailed Description Paragraph Right (25):
The process for the separation and purification of highly unsaturated fatty acids or their derivatives according to the present invention is characterized by using as a carrier the diatomaceous earth having a large pore volume, preferably a pore size of 0.1-10 .mu.m, a specific surface area of 0.5-50 m.sup.2 /g, a pore volume of 1-10 ml/g and a bulk density of 0.1-0.3 g/ml, carrying a specified amount of silver (or silver salt) within the pore of the diatomaceous earth, forming a complex of the raw material to be treated, i.e., the mixture comprising the highly unsaturated acid or the esters thereof, with a silver ion, further using the silver-carried diatomaceous earth within the column, but not in a batch-wise manner, and flowing specified small amounts of solvents sequentially through the column, thereby selectively separating and purifying EPA and DHA or the esters thereof.

CLAIMS:

2. The process of claim 1 wherein the diatomaceous earth has a pore size of 0.1-10 .mu.m, a specific surface area of 0.5-50 m.sup.2 /g and a pore volume of 1-10 ml/g.

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*Original Scientific Paper*

# Influence of Flavonoids on the Stability of an (S)-Hydroxynitrile Lyase from *Hevea brasiliensis*

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Dedicated to the memory of Professor Vera Johanides

**Summary**

The (S)-hydroxynitrile lyase from leaves of the rubber tree *Hevea brasiliensis* (HbHnl) (EC 4.1.2.39) catalyzes the industrially interesting formation of (S)-cyanohydrins from aldehydes or ketones and HCN. The overall yield of the (S)-cyanohydrin is reduced by the non-stereoselective base catalyzed chemical background reaction. To reduce the chemical background reaction, lower pH values (below pH=5) are necessary, but the enzyme in this case is not stable enough. To stabilize the enzyme at these conditions the addition of distinct effectors might be

helpful. The starting point of the stabilizing experiments was the extract of rubber tree leafs. Therefore the leafs were extracted with potassium citrate/phosphate buffer and this extract then chromatographically separated into 5 fractions. The total extract and the fractions thereof were tested for their effect on the enzyme half-life. The extract improved the half-life up to 5 times and one chromatographic fraction increased the stability by 50 %. This fraction was further analyzed and it was found that flavonoids were present. In the next step some pure flavonoids were tested for their effect and it was found that low concentrations of rutin (5-20 ng/mL) and hyperoside (1.5-6 ng/mL) were sufficient to improve enzyme stability up to 50 %. In addition, some synthetic flavonoids (Venoruton®) and monohydroxyethylrutoside) were tested and they also prove this stabilizing effect. The increase of half-life was even higher, almost fivefold in optimal conditions. The increase in the stability is assigned to a chelating effect on the one hand and a structural effect on the other hand. The impact of these effects cannot be presently differentiated.

*Key words*: hydroxynitrile lyase, *Hevea brasiliensis*, stabilization, flavonoids

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# Cassava Research in the Sayre Lab

Cassava is one of the most important food crops in the world, particularly in tropical regions of the world and sub-Saharan Africa. Over 80% of the caloric intake of sub-Saharan Africans is from cassava. Cassava produces cyanogenic glycosides (linamarin) which may be enzymatically broken down to produce cyanide during cell disruption. Typical food processing technologies remove most of the cyanogenic potential from cassava. Under drought or stress conditions, however, short-cut processing procedures may be used resulting in chronic exposure to sub-lethal doses of cyanide. A number of neurological disorders are associated with cyanide exposure from poorly processed cassava. Our lab has been interested in the biochemical and molecular characterization of cyanogenesis in cassava. Recently, we have demonstrated that cassava roots lack an enzyme, hydroxynitrile lyase (HNL), which is required for cyanogen detoxification. We have now generated transgenic cassava plants expressing HNL in roots. Field trials are underway to test the effectiveness of this strategy for reducing cyanide levels in processed foods. In addition, cytochrome P450 enzymes, potentially involved in the synthesis of linamarin, are being expressed in an anti-sense orientation in transgenic cassava plants to reduce cyanogen levels.

A second aspect of our work is altering sink-source

relationships in cassava. The objective is to increase root starch accumulation and shorten the growing season for cassava. A number of genes, which regulate carbohydrate allocation, are being expressed in transgenic cassava so as to increase root starch accumulation.

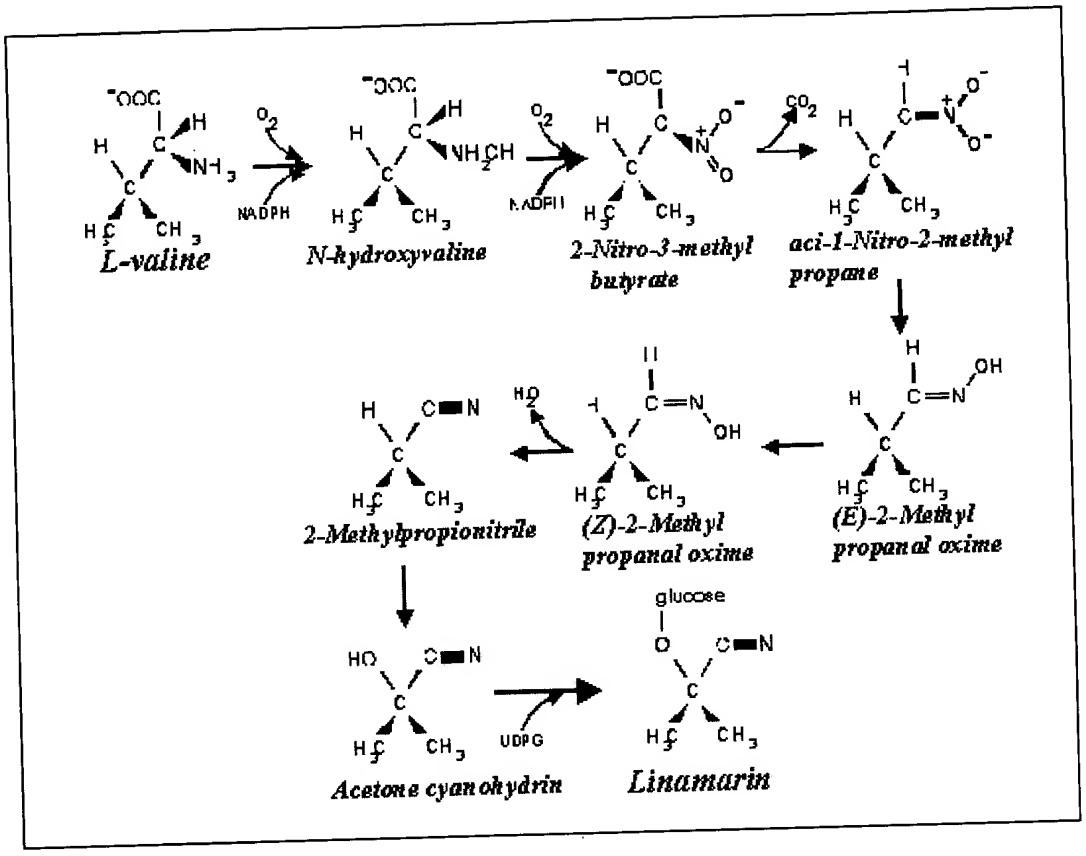
The primary product:

## Cassava in the field

Cassava roots harvested in Africa

The research in the lab falls into several categories:

## Cyanogenesis in Cassava:




Biochemica pathway of Linamarin synthesis

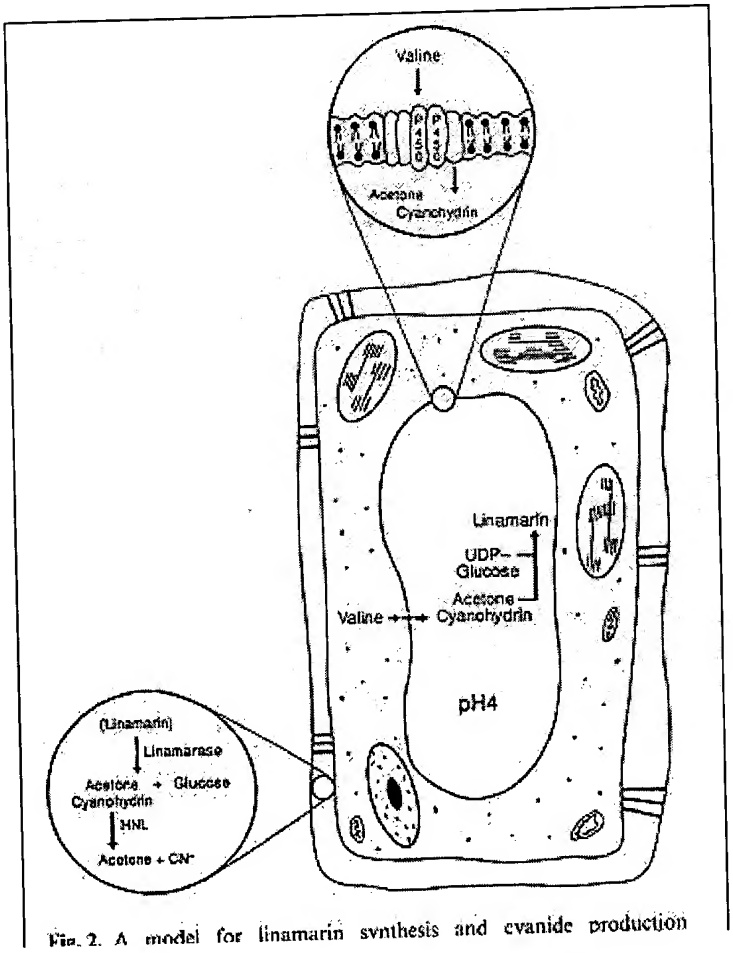
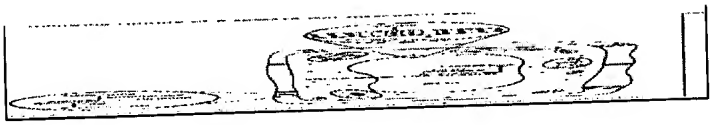



Fig. 2. A model for linamarin synthesis and cyanide production

Biogenesis of cyanide



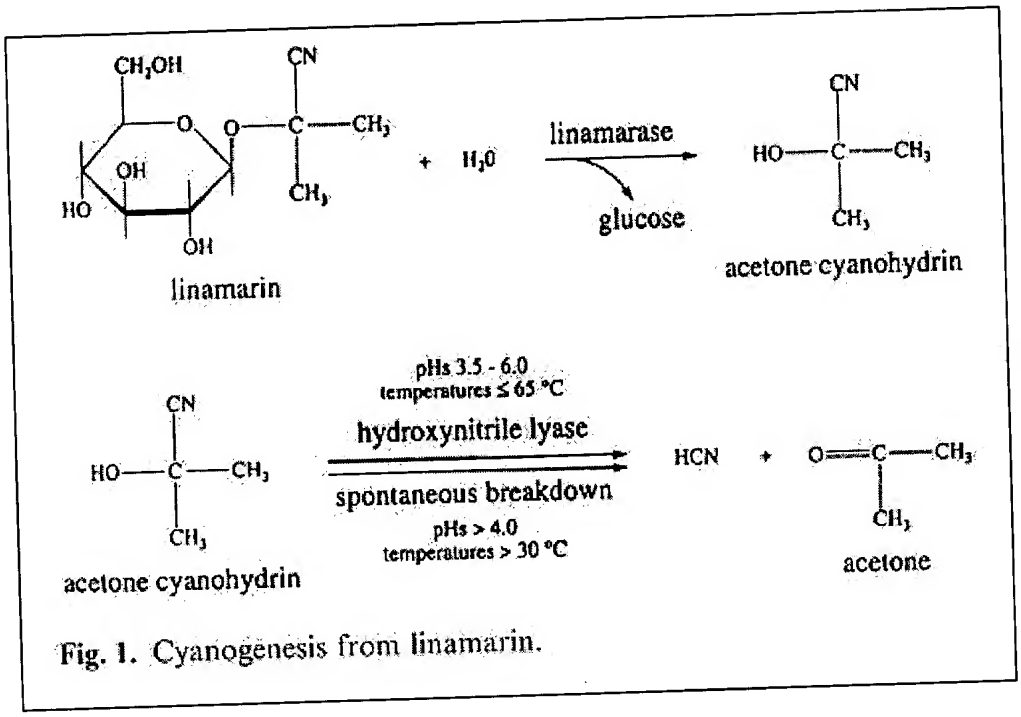
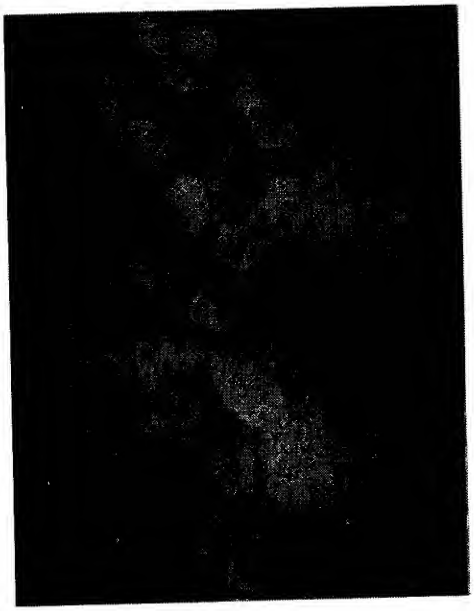



Fig. 1. Cyanogenesis from linamarin.

Breakdown of Linamarin

# Cassava Tissue Culture and Transformation



Induction of somatic embryos in apical leaves of Cassava

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## Publications:

1. McMahon J.M and Sayre RT (1993) Differential biosynthesis of linamarin in low and high cyanide cultivars of cassava. in: Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network; Roca, W.M. and Thro, A.M. eds., pgs. 376-378.
2. Arias-Garzon DI and Sayre RT (1993) Differential inhibition of transient gene expression in cassava root and leaf tissues. in: Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network; Roca, W.M. and Thro, A.M. eds., pgs. 239-243.
3. White WLB and Sayre RT (1993) Partial purification and characterization of hydroxynitrile lyase from cassava. in: Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network; Roca, W.M. and Thro, A.M. eds., pgs. 379-383.
4. Arias-Garzon DI and Sayre RT (1993) Tissue specific inhibition of transient gene expression in cassava (*Manihot esculenta* Crantz) tissues. Plant Science 93:121-130.

5. White W, McMahon J and Sayre RT (1994) Regulation of cyanogenesis in cassava. Acta Horticulturae 375, 69-77.
6. McMahon JM, White W, and Sayre RT (1995) Cyanogenesis in Cassava (*Manihot esculenta* Crantz). Journal Experimental Botany 46:731-741.
7. McMahon J M and Sayre RT (1995) Cyanogenic glycosides: physiology and regulation of synthesis. In: Phytochemicals and Health, D.L. Gustine and H.E. Flores, eds. pgs 75-84.
8. White W and Sayre RT (1995) Characterization of hydroxynitrile lyase for the production of safe food products from cassava. In: Phytochemicals and Health, D.L. Gustine and H.E. Flores, eds. pgs 152-153.
9. Arias-Garzon DI, Sarria R, Gelvin S and Sayre RT (1995) New Agrobacterium tumefaciens plasmids for cassava transformation. in: Proceedings of the Second International Scientific Meeting of the Cassava Biotechnology Network; Roca, W.M. and Thro, A.M. eds., CIAT, Cali, Colombia, no. 150, pgs. 245-256.
10. McMahon JM and Sayre RT (1995) Regulation of cyanogenic potential in cassava (*Manihot esculenta* Crantz). in: Proceedings of the Second International Scientific Meeting of the Cassava Biotechnology Network; Roca, W.M. and Thro, A.M. eds. CIAT, Cali, Colombia, no. 150, pgs. 423-438.
11. McMahon J and Sayre RT (1997) Partial purification of the linamarin synthesizing enzyme complex from cassava. African J. Root Tuber Crops 2: 85-87.
12. McMahon JM and Sayre RT (1998) The Biology and Culture of Cassava Roots. pgs.297-306; In: Radical biology: advances and perspectives on the funcions of plant roots. HF Flores, JP Lynch and D Eissesnstat eds. Am. Soc. Plant Physiol. Publishers.
13. Sayre RT (1998) Recent Advances in the Biochemistry and Molecular Biology of Cyanogenesis in Cassava. Proceedings of the 1st Latin American Conference on Tropical Root Crops, Sao Paulo, Brazil. (in press).
14. White W, Arias-Garzon D, McMahon J and Sayre RT (1998) Cyanogenesis in cassava: The role of hydroxynitrile lyase in root cyanide production. Plant Physiology 116: 1219-1225.
15. Arias-Garcon DI and Sayre RT (2000) Genetic engineering

approaches to reducing the cyanide toxicity in cassava (Manihot esculenta, Crantz).In: Cassava Biotechnology; IV International Scientific Meeting - CBN. Carvalho LJCB, Thro AM and Vilarinhos AD eds. Pgs. 213-221. Embrapa, Brasilia.

## Useful Links for Cassava and Tropical Agriculture:

CIAT- The International Center for Tropical Agriculture

The Cassava page at CIAT

A model describing the growth of cassava (and useful links)

A Lycos search for Cassava links

This page was written by Stuart V Ruffle in 1998 and maintained by Dimuth Siritunga 1999, 2000